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To: Dr. R. N. Ferguson

Date: September 16, 1991

From: D. M. Teng

Subject: Operations Support: Update for the Fourth Quarter, 1991

Nicotine Bioconversion

OBJECTIVE

To determine the feasibility and parameters required to remove nicotine with the microbes in the waste water treatment system.

- A. Determine the parameters required for nicotine bioconversion.

STATUS: Study completed and writing of the special report detailing the work is in progress.

- B. Determine the fate of  $^{14}\text{C}$ -nicotine in a laboratory designed waste water treatment system.

STATUS: Study completed and memo being written. Carbon dioxide accounted for 84% of the label, 15% remained in the reaction liquor and 1% was associated with the solid material in the reaction vessel.

- C. Determine if the nicotine in primary clarifier sludge can be removed through recycling efforts.

STATUS: Initiate study during 4th quarter, 1991.

Microbiological Quality Improvement Program (MOIP)

OBJECTIVE

To eliminate, in all products and processes, microbial activities that lead to unacceptable subjective changes.

- A. Semiworks Make-Pack Study

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**STATUS:** Study completed and memo written. No significant "hot spots" were observed.

### Tobacco Microbiology in Support of Operations

#### OBJECTIVE

In response to unanticipated requests, conduct brief studies with well-defined goals in support of all areas of Operations.

A. Hogshead/Box/Bale Storage Study

**STATUS:** Determine effects on microbial activity (especially mold) of long-term warehouse storage of tobacco in hogsheads, boxes and bales, and in boxes with different liners (with Quality Engineering). Initiated 1986; to continue through 1995.

B. Detailed study of PM80 boxes (with or without plastic sheet on the floor, and with or without Kraft liner at the bottom of the boxes) vs hogsheads using a portion of the 1989 bright and burley tobacco crop.

**STATUS:** Studies completed and memos written. Bright tobaccos in PM80 boxes without Kraft liners showed visible mold at the bottom part of the boxes (both tobacco and the boxes), and elevated OV's.

C. To determine the type and quantity of mold present on tobacco strips and OTM products with the aim of reducing the potential for these materials to experience active mold growth when placed in long-term storage.

**STATUS:** Studies completed and information transferred to appropriate individuals.

D. Leaf Processing Facility (LPF) Study

**STATUS:** During the 1991 bright stemming season, studied the incoming tobacco and devised protocols to sample tobacco on the conveyor belt (or other convenient points) for the zero-time incoming tobacco microbial enumeration. Study completed and memo written. Significant microbial contribution due to crop year, then stemmery and finally grade.

E. Effect of Temperature/RH/OV/Time on Mold Growth

**STATUS:** Ongoing efforts; to date laboratory experiments show tobacco does not mold unless the OV is greater than 25%. Expose bright (whole leaf) tobaccos under different moisture/temperature conditions and determine the time it takes for visible mold growth, or under the conditions which mold growth will not take place (study to be completed in December, 1991).

F. Determine the microbial load in bright tobacco casing during storage.

**STATUS:** Study completed and memo written. No microbes were detected if samples were stored in aseptic containers.

G. Determine the microbial load in burley top casings with and without ethanol (Project Grain).

**STATUS:** Study completed and memo written. Under good sanitary practice both casings were clean.

### Microbiological Methods Development

#### OBJECTIVE

Improve the efficiency, timeliness and accuracy of the measurements of bacterial, yeast and mold populations, and of indicators of biochemical activity, in tobacco and process solutions.

A. Develop a method to reliably sample large volumes of tobacco, i.e., that found in a hogshead/box in support of the storage studies.

**STATUS:** Statistical analysis of the data was completed, sampling protocols were devised, and the memo was written.

B. Study different means of tobacco sample preparation to obtain the optimal condition for extracting tobacco for mold enumeration.

**STATUS:** Study completed, memo written and procedure implemented in the SOP.

- C. Correlate the mold agglutination (MLA) and ergosterol determinations to standard mold plate counts as means of determining mold damage in tobacco.

**STATUS:** Study completed and memo written. The experimental procedures cannot be used to replace the standard plate count method.

- D. Determine feasibility of the "most probable number" (MPN) method for enumeration of bacteria from tobacco.

**STATUS:** Study completed and memo written. This procedure cannot be used in place of the standard plate count method.

### Alternative Preservatives Program

#### OBJECTIVE

To identify tobacco-identical or otherwise natural antimicrobial preservatives to replace or supplement propylparaben in the processing of RL.

- A. Test tetralone (a hop acid known to have antimicrobial properties) from Miller Brewing Company.

**STATUS:** Tetralone was the most active SEL preservative examined in the Phase I screen.

- B. Use multiple combinations of prospective preservatives at concentrations below those necessary for action when present above.

**STATUS:** Studies deferred in favor of other work.

/mps

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